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GEORGIA INSTITUTE OF TECHNOLOGY
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RESEARCH PROJECT INITIATION

Date: June 6, 1974

Project Title: Spectroscopic Characterization of Human Lenses

Project No: G-33-679 (Continuation of G-33-669)

Principal Investigator: Dr. Raymond F. Borkman

Sponsor: Public Health Service

Agreement Period: From June 1, 1974 Until May 31, 1975

Type Agreement: Grant No. 5 R01 EY01138-02

Amount: $27,145 PHS
3,192 GIT Cost-Sharing (G-33-359)
$30,337 Total

Reports Required: Interim Progress with renewal application
Final if project is not to be renewed.

Sponsor Contact Person (s):

Dr. Luigi Giacometti
Extramural Program Director
National Eye Institute
Public Health Service
Bethesda, Maryland 20014

Assigned to: Chemistry

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Patent Coordinator	Other

RA-3 (6-71)

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

Date: June 24, 1977

Project Title: *Spectroscopic Characterization of Human Lenses*

Project No: *G-33-679*

Project Director: *Dr. Raymond F. Borkman*

Sponsor: *DHEW/Public Health Service; National Eye Institute*

Effective Termination Date: *11/30/76*

Clearance of Accounting Charges: *11/30/76*

Grant/Contract Closeout Actions Remaining: *none*

_ Final Invoice and Closing Documents
_ Final Fiscal Report
_ Final Report of Inventions
_ Govt. Property Inventory & Related Certificate
_ Classified Material Certificate
_ Other _____

Assigned to: *Chemistry* (School/Laboratory)

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Project File (OCA)
Project Code (GTRI)
Other _____

CA—4 (3/76)

G-33-679

TERMINAL PROGRESS REPORT

NIH Grant Number EY-01138

"Spectroscopic Characterization of Human Lenses"

June 1, 1973 - November 30, 1976

Raymond F. Borkman (Date) March 25, 1977

Raymond F. Borkman (Principal Investigator)
Associate Professor

School of Chemistry

Georgia Institute of Technology

Atlanta, Georgia 30332

CONTENTS

Scientific Progress Summary

Purpose of Project:

We have available in our laboratory an experimental facility and theoretical competence for investigations in phosphorescence and fluorescence spectroscopy and other optical techniques for the study of polyatomic molecules. The aim of this project was to apply this resource to the study of human and animal lenses and lens proteins in the hope of characterizing cataractogenesis and aging, detecting the incipient incidence of cataracts before they become serious, and ultimately understanding the chemical processes responsible for lens aging and at least some classes of cataracts. The proposed study combined our spectroscopic competence, as physical chemists, with the ophthalmological competence of members of the staff of Emory University, School of Medicine.

The research plan was presented in two parts. In Part I ("*In Vitro* Studies of Human Lenses") we used various spectroscopic techniques to document the physico-chemical changes accompanying clinically defined normal and cataractous lenses, and attempted to interpret the spectroscopic data at the molecular level. In Part II ("*In Vivo* Fluorescence, and Lens and Eye Damage by UV Radiation") we spectroscopically analyzed lenses previously exposed to UV radiation and compared these results with results from normally aging lenses.

In the discussion which follows, we reiterate the original specific questions raised in our proposal and indicate the progress made in answering these questions.

The work is being continued in collaboration with Dr. Sidney Lerman of Emory University and is supported by NIH Grant EY-01575 to Emory University.

Results and Discussion

Question I-1 : Can different varieties of clinically defined cataracts be distinquished from each other and from normal lenses of various ages by spectroscopic analysis of intact lenses?

(a) We have succeeded in recording UV-excited fluorescence spectra of intact human and animal lenses. Previous reports have dealt mainly with spectra of lens extracts, homogenates, etc.

(b) We have shown that there are at least two important spectral regions in lens fluorescence studies: 360 nm excitation/440 nm emission and 290 nm excitation/332 nm emission, see Fig. 1.

(c) We have demonstrated that the intensity of the 360/440 nm fluorescence in human lenses is strongly correlated with age and with certain types of cataracts (Fig. 2).

(d) We have detected an additional non-tryptophan fluorescence band in intact human lenses. This fluorescence is characterized by 435 nm excitation and 520 nm emission and its intensity is also strongly correlated with age and with cataract type. These results are summarized in Fig. 3.

Question I-2 : Can a well-collimated spectroscopic excitation beam be used to probe differences between clear and opaque (or colorless and pigmented) regions of an intact, cataractous lens?

We have not investigated this problem as yet.

Question I-3 : Given that cataracts are a manifestation of altered protein structure in the lens can these protein changes be detected spectroscopically? In intact lenses? In lens protein fractions?

This question has been taken up by Dr. Nai-Teng Yu of this department who is using Raman spectroscopy to probe lens protein structure.

Question I-4 : Can the characteristic tryptophan-like fluorescence and phosphorescence of human lenses be shown to originate primarily from a particular lens protein fraction: α, β, γ-crystallins, albuminoid, peptide, or from free tryptophan?

(a) We have provided evidence that the 290/332 nm fluorescence originates from protein bound tryptophan residues and that most of these residues exist in a non-polar hydrophobic environment in the ocular lenses of humans and various other species.

(b) We have begun an analysis of lens protein fluorescence using the method of Burstein et al. This method is based on precise measurement of tryptophan fluorescence parameters: emission maximum, band width, and lifetime. Our results for intact young rat lenses (λ_{max} = 331 nm, $\Delta\lambda$ = 47 nm, τ_F = 2.3 nsec) lead to the conclusion that the majority (> 90%) of the tryptophan residues in rat lenses belong to TYPE I class, typical of buried tryptophans in the protein chymotrypsinogen. Typical tryptophan fluorescence decay data are shown in Fig. 4.

Question I-5 : In cataracts, are there new, spectroscopically detectable, chemical species (pigments) present? What are the chemical identities of these pigments?

(a) Our fluorescence measurements have confirmed the presence of high concentrations of fluorescent pigments in nuclear cataracts.

(b) The identities of these pigments have not been elucidated with certainty. We have suggested the use of fluorescence lifetimes as an additional parameter for characterizing these pigments (fluorogens) in intact lenses.

(c) There is preliminary evidence that these pigments are identical to materials generated photochemically in the lens (see below) and as such are likely to be tryptophan degredation products.

Question I-6 : Can one obtain spectroscopic evidence for the destruction of particular amino acids in aging or cataractous lenses?

We have preliminary fluorescence evidence for loss of tryptophan in very old human lenses and in severe nuclear cataracts.

Questions I-7 : Are the light emitting chromophores in the lens specifically bound and oriented within the macrostructure or are they relatively free to rotate and translate?

The answer to this requires fluorescence polarization experiments which have not yet been performed.

Question II-1 : What are the effects of prolonged UV radiation on cultured human and animal lenses?

(a) We have demonstrated that exposing young human or animal lenses in vitro to monochromatic light generates fluorescence (360/440 nm) identical to that which occurs naturally in old lenses and nuclear cataracts, Fig. 5.

(b) We have measured the dependence of the rate of production of fluorescent material on irradiation wavelength and have thereby

calculated the "action spectrum" for production of 360/440 nm fluorescence in young rat lenses. This reveals the wavelengths of UV light most effective in damaging the ocular lens in vitro, Fig. 6.

(c) We have performed ESR measurements on human and rat lenses irradiated in situ in an ESR cavity and have observed free radical signals at g = 2.007, Fig. 7. In addition to confirming the presence of free-radicals in UV-irradiated lenses, these ESR studies also correlated with our fluorescence studies in respect to characterizing differences between young and old lenses and normal versus cataractous lenses, Fig. 8.

Question II-2 : How are the above effects altered by changes in the incubation medium?

(a) We have observed that addition of 3-aminotriazole to the incubation medium greatly enhances the rate of UV-induced damage. This is presumably due to catalase inhibition.

(b) We have confirmed both that addition of penicillamine to the culture medium greatly suppresses the UV-induced production of fluorescent material, and that lenses pre-incubated in the presence of penicillamine fail to show ESR activity when irradiated in the ESR cavity, Figs. 5, 7.

Question II-3 : What molecular changes occur in UV irradiated lenses and lens protein extracts?

(a) We have measured the action spectrum for UV destruction (photo-oxidation) of tryptophan in aqueous solution. The data indicate that this photochemical reaction proceeds via photoionization of tryptophan, at least for irradiation wavelengths less than 280 nm, and possibly also for longer wavelengths.

(b) Comparison of the tryptophan and lens action spectra, Fig. 6, has led us to two conclusions. First, that tryptophan is an important light absorbing species in lens photobiology in vitro. Second, that lens photobiology (especially production of fluorescent compounds) is initiated by photochemical alteration of tryptophan residues in lens protein.

Question II-4 : What is the molecular mechanism responsible for such changes?

The results cited on the effects of 3-aminotriazole and penicillamine in the medium, the action spectral data, and the ESR results all point to a free-radical mechanism in UV-induced lenticular damage.

Question II-5 : Can fluorescence parameters be measured in vivo. Preliminary experiments have been performed in which fluorescence spectra from rabbit eyes in vivo are compared with spectra of whole globes in vitro and with separate cornea, aqueous, and lens. The results indicate that the fluorescence from cornea and aqueous is ten times weaker than that from the lens, and therefore meaningful in vivo lens spectra may be feasible in the future.

Figure 1

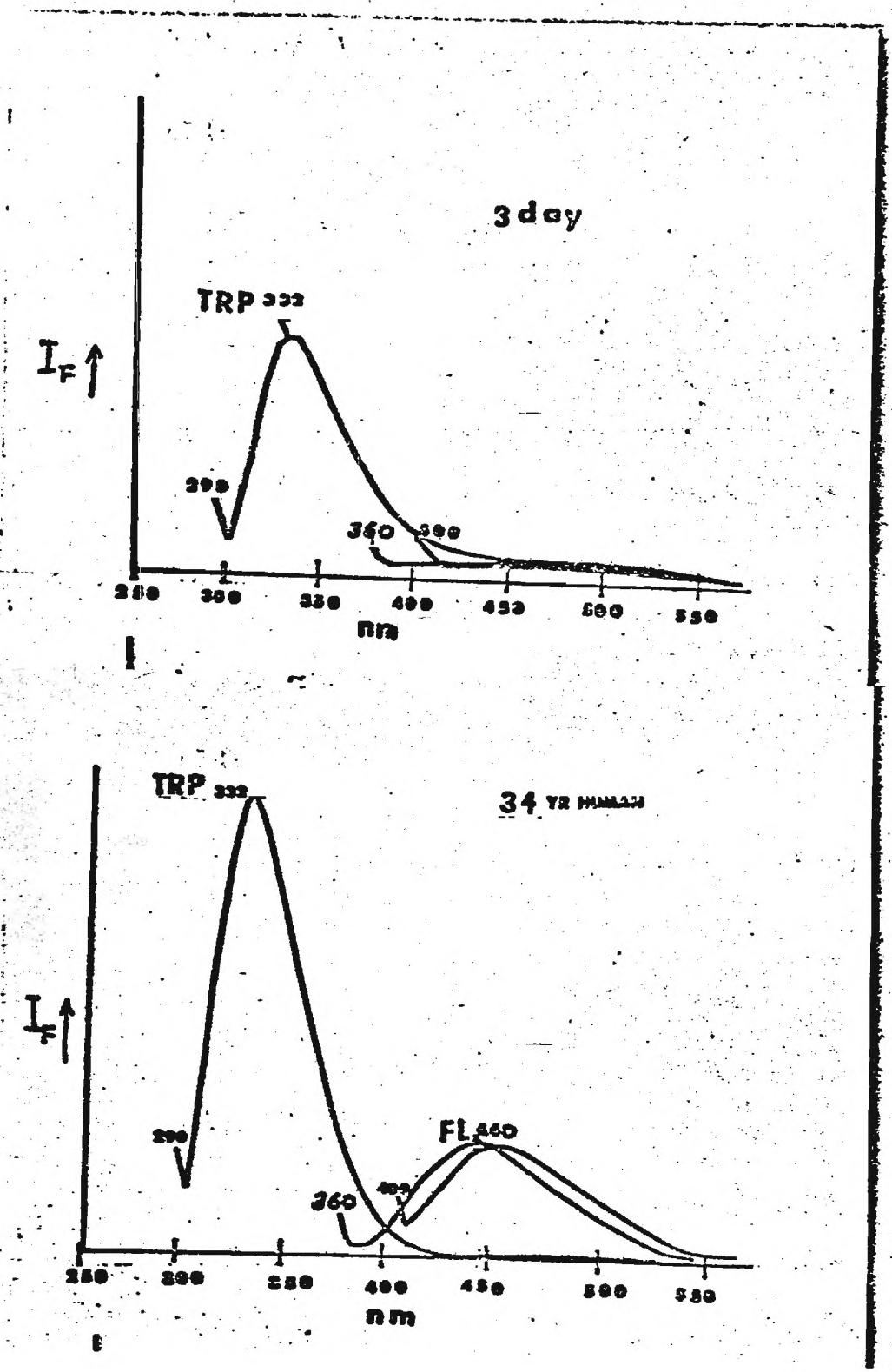
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Figure 2

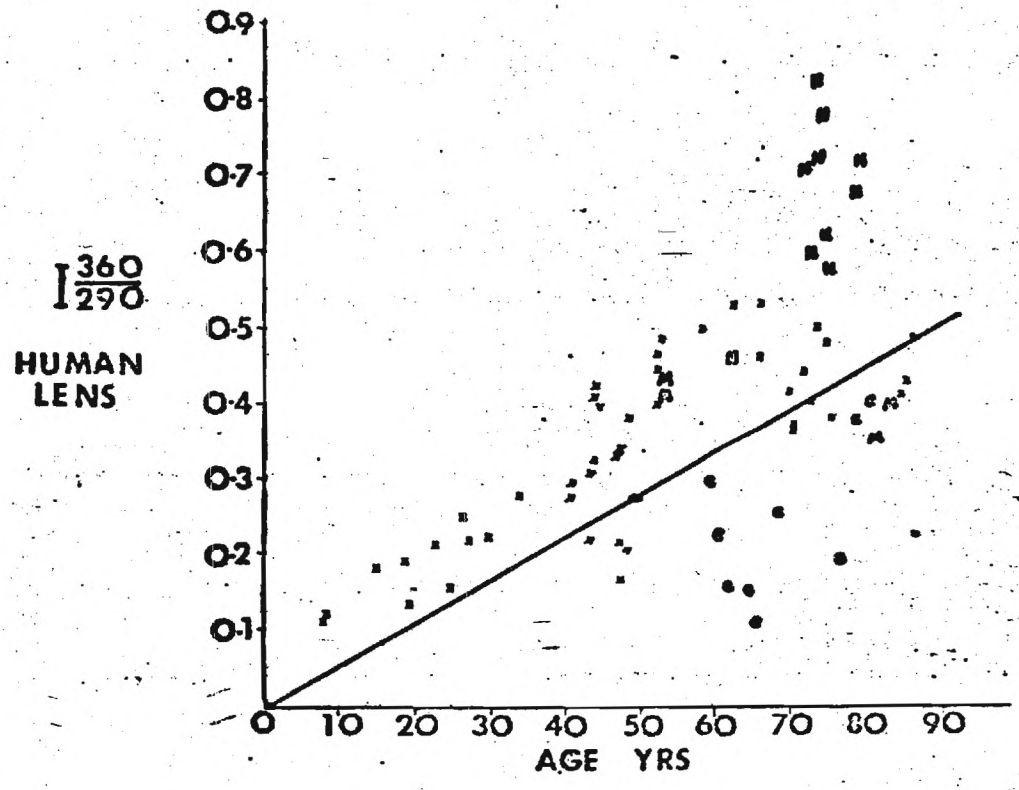
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Figure 3

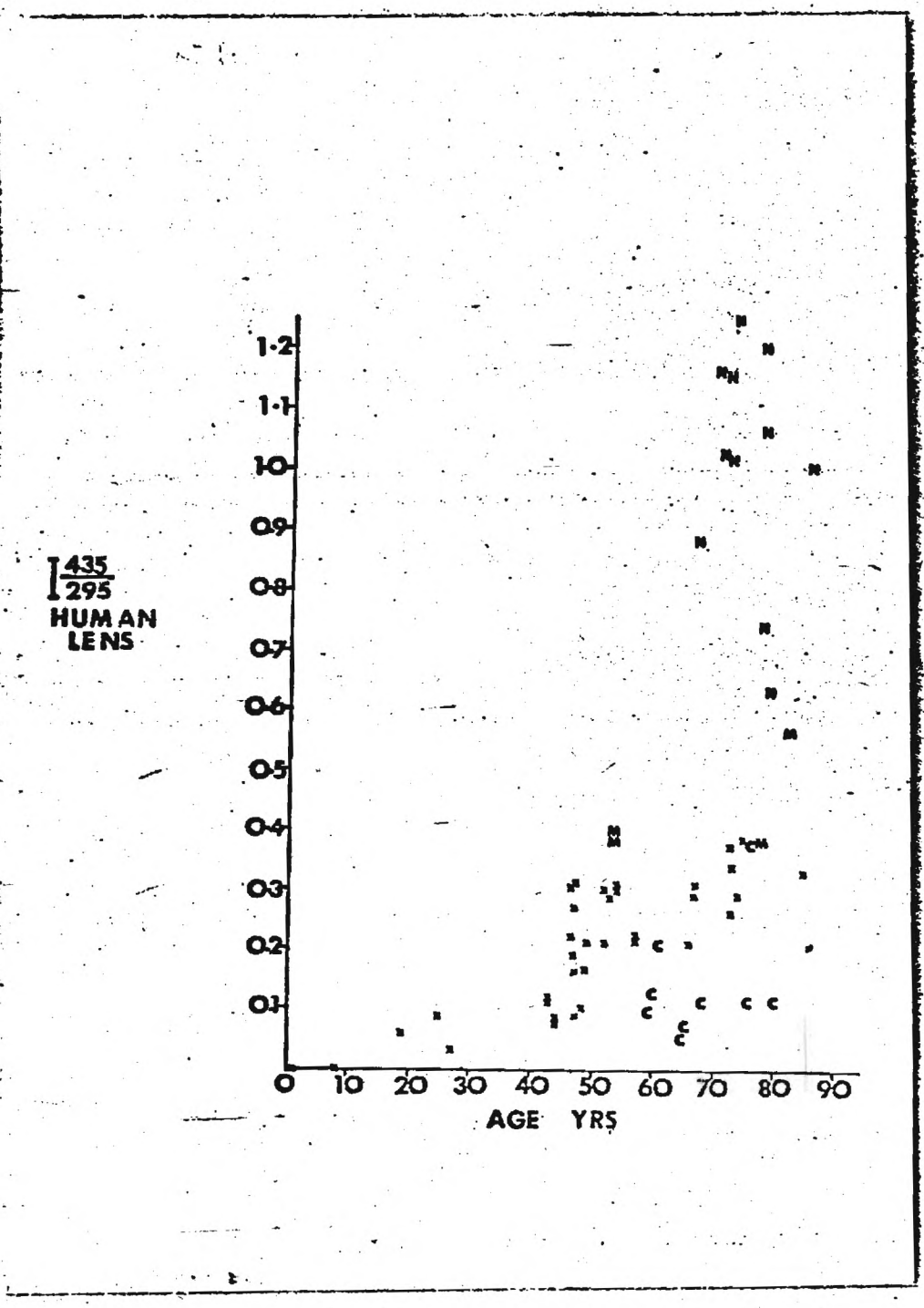
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Figure 4

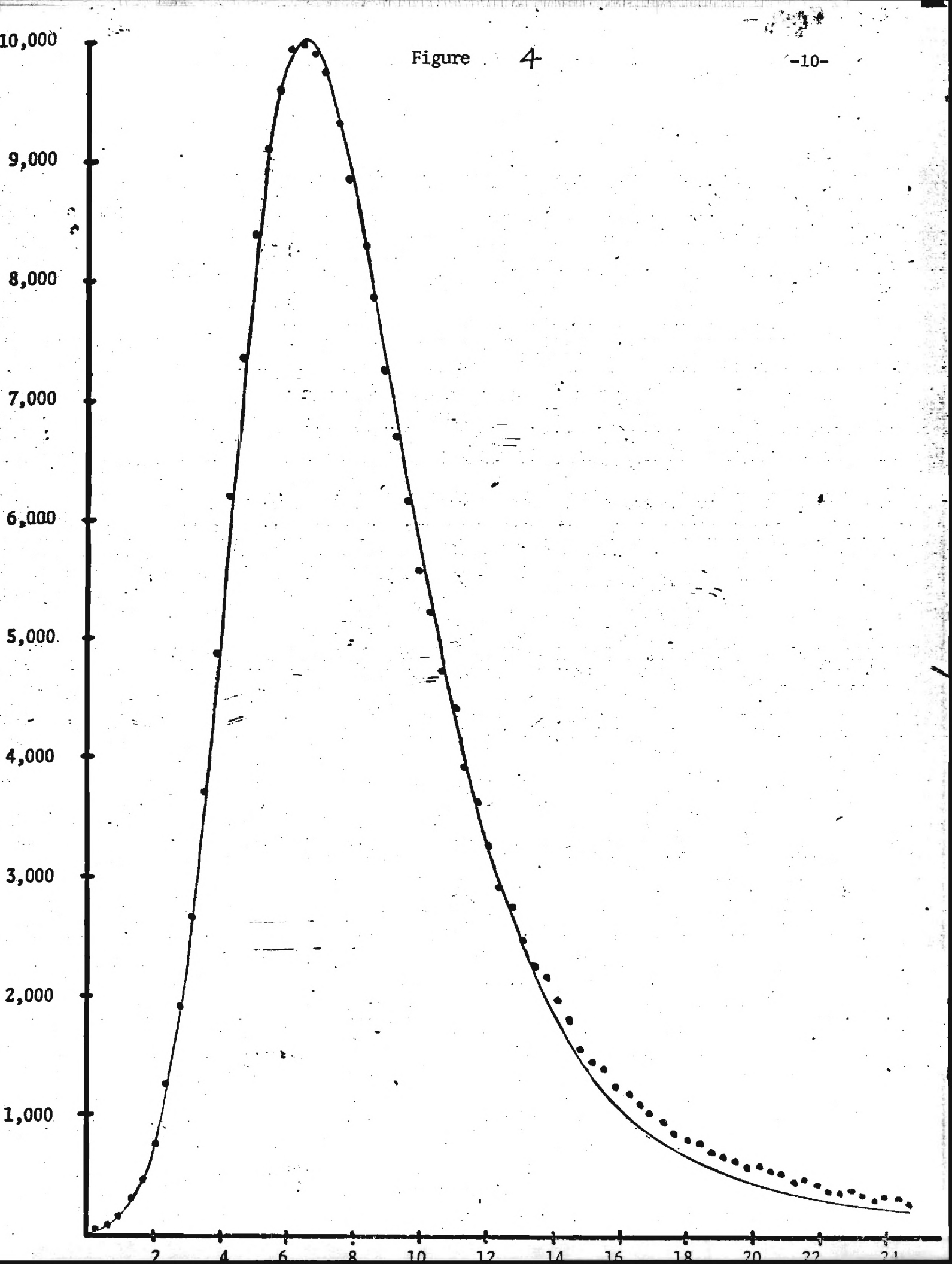
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Figure 5

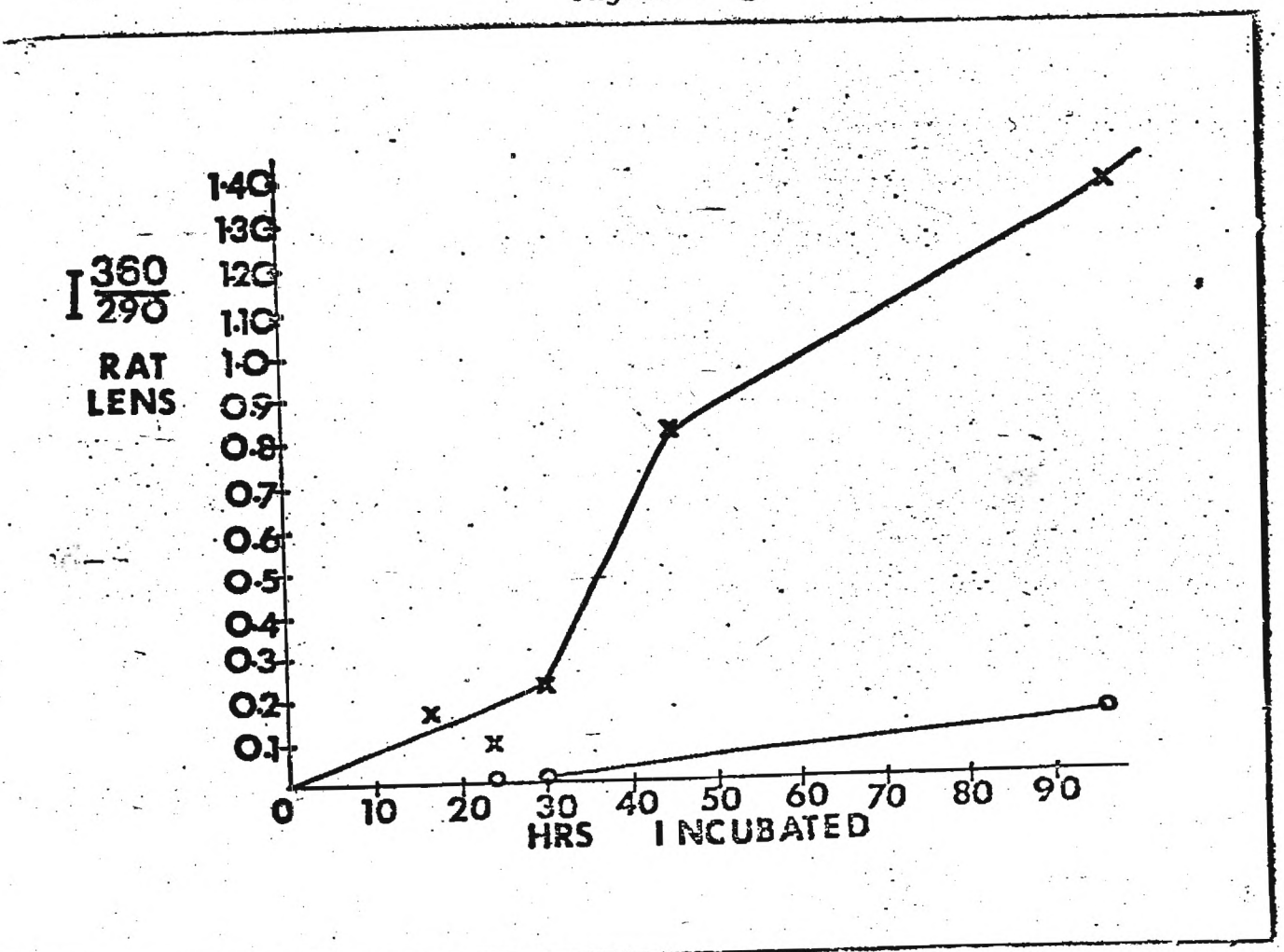
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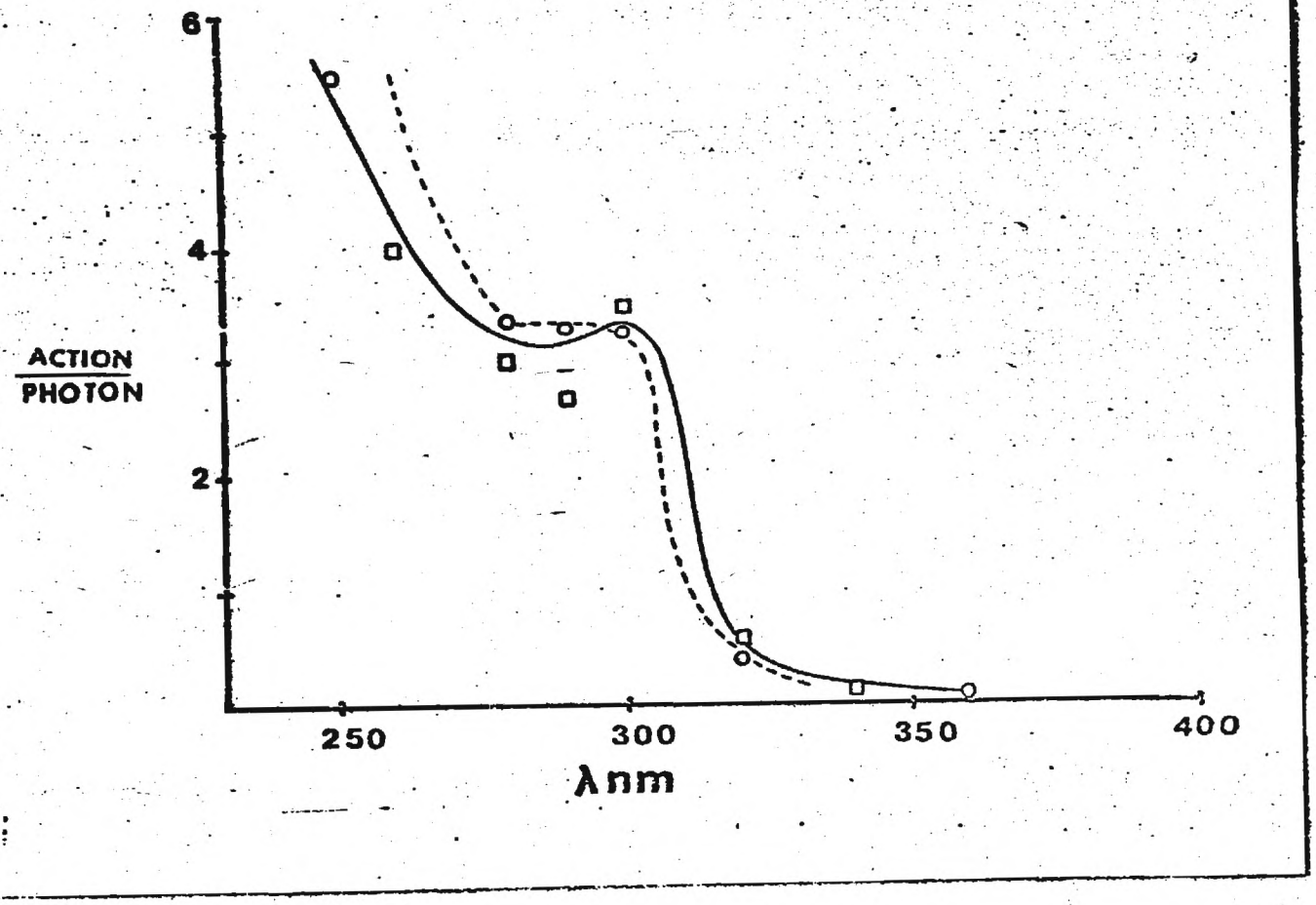
Figure 6



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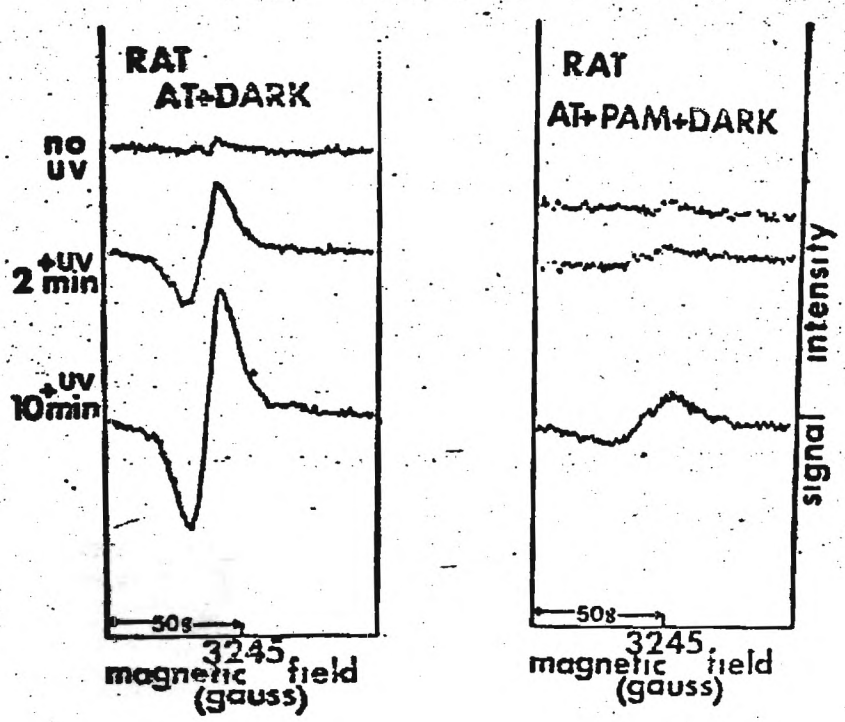
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Figure 8

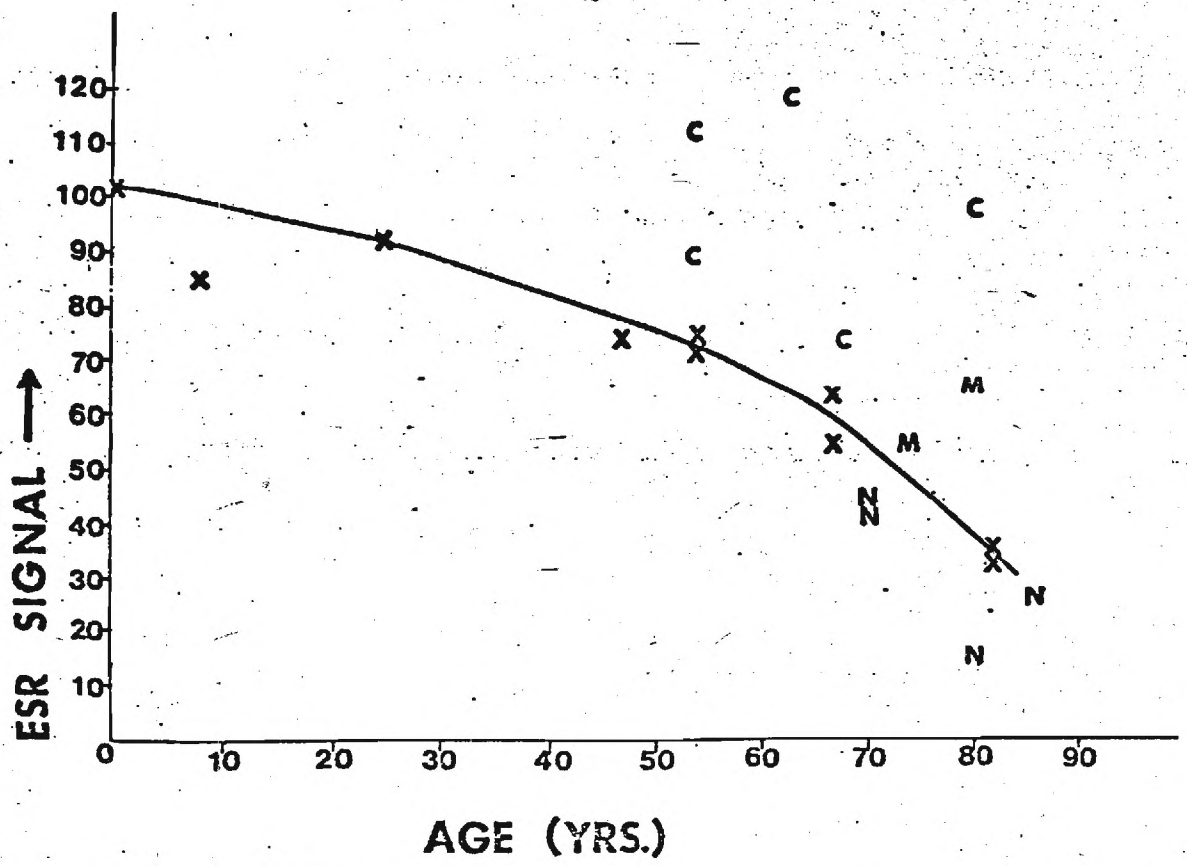

FIGURE CAPTIONS

Figure 1: Fluorescence spectra of 3 day and 34 year old intact normal human lenses showing TRP 332 fluorescence and 440 nm fluorogen.

Figure 2: $I\frac{360}{290}$ ratios representing fluorescence intensity of $\frac{360}{440}$ nm fluorogen versus TRP fluorescence $\frac{290}{332}$ nm. $I\frac{360}{290}$ ratio shows age related increase in normal human lens (x); marked increase in brown nuclear cataracts (N); relatively normal levels in cortical cataracts (c) and high/normal values in mixed (cortical and nuclear) cataracts (M).

Figure 3: $I\frac{435}{295}$ ratios representing fluorescence intensity of $\frac{435}{520}$ nm fluorogen versus TRP fluorescence $\frac{295}{332}$ nm (295 and 435 interference filters employed in order to minimize light scattering in cortical cataracts). Symbols, X represents $I\frac{435}{295}$ values in normal lens; N (nuclear cataracts), C (cortical cataracts) and M (mixed nuclear and cortical cataracts).

Figure 4: Decay kinetics for tryptophan fluorescence from a 2 month old rat lens. Plotted points represent the experimental data. The solid line is the calculated decay assuming a fluorescence lifetime of 2.3 nsec.

Figure 5: $I\frac{360}{290}$ ratio in 2 month old rat lens incubations containing 10 mM 3-aminotriazole (AT) and exposed to UV radiation for varying periods of time (x). o----o represent similar experiments in which 10 mM D-penicillamine was added to the incubation mixture prior to UV exposure.

Figure 6: Action spectrum (fluorogen produced per incident photon) for fluorogen production in incubated rat lenses. The ○ and □ symbols represent the results of two independent experiments. The dashed curve represents the predicted rat lens action spectrum calculated from data on free tryptophan in aqueous solution.

Figure 7: ESR signal from normal 2 month old rat lens incubated with AT in the dark compared with signal obtained from contralateral lens incubated with AT and PAM in the dark (incubation time = 6 hours).

Figure 8: ESR signal intensity (expressed as arbitrary units; 1 day = 100) determined on 3 mm cores of normal lenses (X) compared with ESR signals obtained from cortical (C), nuclear (N) and mixed (M) cataracts.

List of Publications and Proposed Publications

1. Lerman, S., Kuck, Jr., Borkman, R., and Saker, E., "In Vivo and In Vitro UV Induced Lens Fluorogen(s) in various Species," ARVO Annual Meeting, 1975.

2. Lerman, S., Kuck, J ., Borkman, R., and Saker, E., "Fluorescence Spectroscopy of the Aging Human Lens," ARVO Annual Meeting, 1975, p. 84.

3. Lerman, S., Kuck, J. F., Borkman, R., and Saker, E., "Acceleration of the Aging Parameter (Fluorogen) in the Ocular Lens," Ann. Ophthal., 8:558-562, 1976.

4. Lerman, S., Kuck, J. F., Borkman, R., and Saker, E., "Induction, Acceleration and Prevention (In Vitro) of an Aging Parameter in the Ocular Lens," Ophthalmic Res., 8:213-226, 1976.

5. Lerman, S., Borkman, R. F., Yu, N. T., Kuck, J. F., and Saker, E., "Spectroscopic Studies on Normal and UV Accelerated Aging in the Lens," ARVO Annual Meeting, 1976, p. 6.

6. Borkman, R. F., Dalrymple, A., and Lerman, S., "UV Action Spectrum for Production of a Fluorogen in the Ocular Lens,"ARVO Annual Meeting, 1976, p. 113.

7. Lerman, S., Borkman R., Yu, N.T., and Kuck, J., "UV Radiation in Lens Aging and Cataract Formation," Second International Congress of Eye Research, Jerusalem, Israel, 1976.

8. Borkman, R., Dalrymple, A., and Lerman, S., "UV Action Spectrum for Fluorogen Production in the Ocular Lens," Second International Congress of Eye Research, Jerusalem, Israel, 1976.

9. Lerman, S., and Borkman R., "Spectroscopic Evaluation and Classification of the Normal, Aging and Cataractous Lens," Ophthalmic Res., (in press, 1977)

10. Borkman, R. F., Dalrymple, A., and Lerman, S., "Ultraviolet Action Spectrum for Fluorogen Production in the Ocular Lens," Photochem. and Photobiol., (in press, 1977).

11. Borkman R. F., "UV Action Spectrum for Tryptophan Destruction in Aqueous Solution," Photochem. & Photobiol. (in press, 1977).

12. Borkman, R. F., and Lerman, S., "Evidence for a Free Radical Mechanism in Aging and UV-Irradiated Ocular Lenses," Exp. Eye Res., (submitted, 1977).

Project Personnel

R. F. Borkman, Principal Investigator, Associate Professor of Chemistry, Georgia Institute of Technology, June, 1973 - November 1976.

S. Lerman, Collaborator, Professor of Ophthalmology, Emory University, June 1974 - November 1976.

J. F. R. Kuck, Collaborator, Associate Professor of Ophthalmology, Emory University, June, 1973 - November, 1976.

E. Saker, Postdoctoral Research Associate, School of Chemistry, Georgia Institute of Technology, June, 1973 - June, 1976.

J. Robertson, Postdoctoral Research Associate, School of Chemistry, Georgia Institute of Technology, September, 1973 - September, 1974.

J. Tassin, Graduate student, School of Chemistry, Georgia Institute of Technology, September, 1975 - November, 1976.

C. Stoner, A. Dalrymple, Lab Technicians, School of Chemistry, Georgia Institute of Technology, June,1975 - December, 1975.

APPENDIX

2 Reprints Attached

4 Reprints to be Forwarded Later